INTERACTION OF PYRIDOSTIGMINE WITH THE 5-HT_3 RECEPTOR ANTAGONIST ONDANSETRON IN GUINEA PIGS

BR. Capacio, CE. Byers, RL. Matthews,
DR. Anderson and JC. Anders

Applied Pharmacology Branch
U.S. Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425

ABSTRACT

Serotonin receptor subtype three ($5HT_3$) antagonists, such as the drug ondansetron (OND), have been developed as effective anti-emetic compounds. The purpose of this study was to assess the drug interactions of OND (10, 20 and 30 mg/kg) with the organophosphorus pretreatment compound pyridostigmine (PYR; 0.94 mg/kg) after simultaneous oral administration to guinea pigs. Compatibility was assessed by determining 1) OND pharmacokinetics in the absence (Phase 1) and presence (Phase 2) of pyridostigmine (PYR) and 2) PYR-induced acetylcholinesterase (AChE) inhibition kinetics in the absence (Phase 1) and the presence (Phase 2) of OND. AChE inhibition was examined because it has been shown to be an indicator of PYR efficacy against OP-induced lethality. The pharmacokinetics of OND alone and in the presence of PYR were linear and best described by a one-compartment model with first-order absorption and elimination rate kinetics. For OND 30 mg/kg the K_{10} was found to be significantly smaller in Phase 2 than Phase 1 ($p < 0.05$). The difference in K_{10} relative to Phase 1 (Phase 2 - Phase 1/Phase 1) expressed as a percentage was calculated to be -42%. The 20 mg/kg dose demonstrated significant differences between Phase 1 and Phase 2 for the parameter estimates K_{01}, K_{10}, C_{max}, $K_{01}HL$ and AUC ($p < 0.05$). The differences were calculated to be 84.0, -35.5, 33.0, 50.5 and 43.0% respectively. There were no significant differences in the mean parameter estimates between Phase 1 and Phase 2 for ondansetron 10 mg/kg. For Ache inhibition, OND alone did not provide unequivocal indications of RBC AChE inhibition. However, the simultaneous administration of OND (10 and 20 mg/kg) and PYR produced greater AChE inhibition than either compound alone at 30 through 240 minutes (10-20%; $p < 0.05$). The significant effects on the pharmacokinetic parameters and AChE inhibition were eliminated by administering OND and PYR 150 minutes apart. The data from this study suggest that in the guinea pig,

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PYR may interfere with the metabolic elimination of OND. Secondly, the effects of OND on $5HT_3$ gut receptors (i.e., enhanced motility and increased PYR absorption) may be responsible for the increased PYR-induced AChE inhibition we have noted. At this point the clinical significance of these findings is not clear.

INTRODUCTION

It has been suggested that 5-hydroxytryptamine (5-HT) may be involved in mediating emetic responses (1-2). A number of specific 5-HT receptor subtype three antagonists ($5HT_3$) such as the compound ondansetron (OND) have been identified as useful in the treatment of chemical- or radiation-induced nausea and vomiting (3-4). Furthermore, it offers the advantage of not possessing sedative/hypnotic (5) or extrapyramidal effects associated with other anti-emetic compounds such as metoclopramide (6).

Pyridostigmine (PYR) has been utilized as the drug of choice for pretreatment against OP-induced lethality (7). However, the compatibility of PYR with other pretreatment compounds such as OND has not been addressed. The prospect of simultaneous exposure to radiation and organophosphorus (OP) compounds has prompted the assessment of potential interactions between the respective pretreatment compounds.

The purpose of this study was to assess the compatibility of the novel anti-emetic $5-HT_3$ antagonist OND with the OP pretreatment PYR. Interactions between PYR and OND were assessed in two ways. The effects of PYR on OND pharmacokinetics and, conversely, the effects of OND on PYR-induced AChE inhibition were investigated. Herein we report the results of these studies.

EXPERIMENTAL

Animals

Male guinea pigs Crl:(HA)BR Hartley COBS® weighing 650-800 g were utilized for all experiments. They were quarantined on arrival and observed for evidence of disease for five days prior to experimental use. They were maintained under an American Association for Accreditation of Laboratory Animal Care program, in polycarbonate cages on hardwood chip contact bedding with a complete cage change two times per week. They were provided commercial Certified Guinea Pig ration as appropriate, and tap water ad libitum. Since all drugs were administered orally, the animals were fasted overnight (18 hours) prior to the day of experimentation. During this period they were allowed tap water ad libitum. The guinea pig holding room was maintained at 21 ± 2°C with 50 ± 10% relative humidity using at least 10 complete air changes per hour. They were on a 12-hour light/dark full spectrum fluorescent lighting cycle with light provided between 0600 and 1800 hours.

Drugs

All drugs were administered between 0800 and 01000 hours, via oral gavage tube (18G X 3", Popper and Sons, Inc.). The compounds were made up in sterile water for injection, USP (Abbott Laboratories, North Chicago, IL). Pyridostigmine bromide was obtained from Walter Reed Army Institute of Research, Washington DC. Ondansetron was obtained in the form of oral tablets containing 8 mg of active ingredient. An appropriate number of tablets were triturated and dissolved in a volume of sterile water for injection to make gavage solutions which would deliver 10, 20, or 30 mg/kg of OND. In all experiments the animals received dosages of drugs in a volume equivalent to 5 ml/kg. DL-(1-^{14}C)-acetyl-β-methyl choline bromide was obtained from Amersham

(Arlington Heights, Ill). All chemicals and solvents for high performance chromatography (HPLC) procedures were of HPLC grade or higher quality.

Experimental Design and Procedures

For all experiments, the drugs were administered orally. The dose of PYR in all experiments was 0.94 mg/kg. The effects of PYR on OND pharmacokinetics and the effects of OND on PYR-induced AChE inhibition were investigated by carrying out two experimental paradigms (Phase 1 and Phase 2). Reversible AChE inhibition by compounds such as PYR has been shown to play a critical role in protection against OP lethality; therefore this parameter was utilized as the index of PYR activity (8). In Phase 1, pharmacokinetic parameters and AChE inhibition time-course profiles were determined for OND (10, 20 and 30 mg/kg). The pharmacokinetic estimates included apparent volume of distribution (Vd), absorption rate constant (K_{01}), elimination rate constant (K_{10}), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half-lives associated with absorption and elimination ($K_{01}HL$ and $K_{10}HL$ respectively) and area under the concentration-time curve (AUC). The animals in this phase received either water or anti-emetic. The water control group was utilized to establish the procedural effects on AChE activity as a function of time. In Phase 2, OND (10, 20 and 30 mg/kg) and PYR were given together (within 30 seconds) and the pharmacokinetic estimates and AChE inhibition time-course profiles were again determined. For this phase the appropriate dose of drug was delivered in a volume of 2.5 ml/kg such that the total volume to an animal was 5 ml/kg. Additionally, a third phase (Phase 3) was carried out. This experiment followed two paradigms: 1) administration of OND followed 150 minutes later by PYR, with subsequent determination AChE inhibition kinetics and 2) administration of PYR followed 150 minutes later by OND, followed by the determination of pharmacokinetics.

Blood Collection

Serial blood samples (approximately 0.350 ml) were collected at 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 minutes after dosing. Blood was collected by the toe nail clip method (9) in heparinized capillary Microvette® tubes (Starstedt, Newton, NC) and placed on ice. Immediately, 2 X 25 μl aliquots of whole blood were removed and used for the AChE assay.

Sample Preparation for Ondansetron Determinations

Plasma was obtained by centrifuging whole blood in the capillary tubes at 2000 x g for 15 minutes at 10°C. The resulting plasma was then transferred to Centricon-10 (molecular weight cutoff 10,000) microconcentrator tubes (Amicon Corp., Danvers, MA) and centrifuged at 7,000 x g at 10°C for 70 minutes with a fixed angle SM-24 rotor. Approximately 60 μl of filtrate was recovered for analysis by HPLC. All samples were analyzed directly by HPLC within several hours of preparation and were refrigerated until analysis.

Plasma Ondansetron Determinations

The samples were analyzed utilizing the HPLC method of Culthup and Palmer (10). OND plasma concentrations were determined with a computer controlled BAS 200 HPLC with data analysis software (Bioanalytical Systems, Inc., West Lafayette, IN). A control sample from pooled plasma (spiked) was analyzed with the unknowns each experimental day. Briefly, 10 μl of filtered plasma was injected onto a Spheri-5 silica column (100 x 4.6 mm, 5 μ; Applied Biosystems, Inc., San Jose, CA) with a Carnegie Medicin CMA 200 autosample injector equipped with a 20 μl sample loop (Carnegie Medicin, Stockholm, Sweden). The detector response sensitivity was set at 0.005 absorbance units full-scale at a wavelength maximum of 305 nanometers. The maximum wavelength

was determined employing an ultraviolet (UV)/visible scanning spectrophotometer (Varian 100 scanning spectrophotometer). Mobile phase consisted of 40% acetonitrile, 60% sodium acetate 0.025 M, pH 4.2 and was delivered at a rate of 1 ml/minute. The plasma concentration-time data derived from this analysis was used to determine pharmacokinetic parameters.

Pharmacokinetic Analysis

Plasma concentration-time data were found to best fit a bi-exponential equation representing a one-compartment model with first-order absorption and elimination. It was found that the data could not be adequately described by a two compartment model. Pharmacokinetic parameter estimates (Vd, K_{01}, K_{10}, T_{max}, C_{max}, $K_{01}HL$, $K_{10}HL$ and AUC) were determined utilizing PCNONLIN (statistical Consultants, Inc., version 3.0, 1989) non-linear regression software. Initial estimates for each were determined from curve stripping computer program JANA (Statistical Consultants, Inc., 1986). Range limits were set to constrain the time constants (K_{01} and K_{10}). After the solution was found, the actual values that produced the optimum fit were checked to verify that they were not at either limit of the range over which they were allowed to vary. Each concentration-time profile was characterized in terms of the parameter estimates that produced the best fit to the standard model. For each dose within a phase the mean parameter estimates were calculated and are reported as the mean ± standard error of the mean.

RBC Acetylcholinesterase Assay

The radiometric method of Talbot (11) was utilized for these determinations. This method utilized DL-(1-^{14}C)-acetyl-β-methyl choline as the substrate and, therefore, was specific to RBC AChE activity. The assay was performed immediately upon acquisition of the 2 X 25 μl whole blood aliquot samples. For any given animal, the T = 0 sample was utilized as control AChE activity and all subsequent time points were expressed as a percent of that activity. All samples were counted in a Packard 2500 TR liquid scintillation counter.

Statistical Analysis

For pharmacokinetic data, regression analyses, mean parameter estimates and standard error calculations were performed with Number Cruncher Statistical System (NCSS) software, version 5.03 (1991, Jerry L. Hintze, Kaysville, UT, USA). Mean pharmacokinetic parameter estimates were compared utilizing SAS. For these analyses a two factor general linear model was utilized. Further GLM analyses were performed for each phase to assess dose differences, and for each dose to assess phase differences. A post hoc Duncan's multiple range test was used when significant differences were detected. Significance was considered at a level of $p < 0.05$. For AChE data, NCSS was employed to perform repeated measures analysis of variance to detect significant differences ($p < 0.05$) in treatment groups compared to appropriate controls. A Newman Keul's post hoc test was used to examine significantly different treatments at each time point.

RESULTS

Pharmacokinetics

Ondansetron pharmacokinetics, alone (Phase 1) and in the presence of PYR (Phase 2), were characterized by a one-compartment model with first-order absorption and elimination. The concentration-time profiles for both phases could be described by the following equation.

$$C(t) = \frac{D}{Vd} \cdot \frac{K_{01}}{K_{01} - K_{10}} \left(e^{-K10t} - e^{-K01t}\right)$$

where C = plasma concentration (ηg/ml), t = time (min), D = dose (ηg/kg). Vd = apparent volume of distribution (ml/kg), K_{01} = rate constant of absorption ($minutes^{-1}$), and K_{10} = rate constant of elimination ($minutes^{-1}$). Pharmacokinetic parameter estimates for Phases 1 and 2 are presented in Table 1. Analysis of variance detected significant differences for C_{max} and AUC in Phases 1 and 2. Within each phase, post hoc test results indicated that the parameter estimates for C_{max} and AUC were significantly different ($p < 0.05$) for each dose. Additionally, there was a linear relationship for C_{max} (correlation coefficients = 0.9626, Phase 1; 0.9990, Phase 2) and AUC

Table 1: Comparison of Phase 1 and Phase 2; Mean Pharmacokinetic Parameters.

ONDANSETRON (30 mg/kg)		
PARAMETER	**PHASE 1**	**PHASE 2**
Volume (l/kg)	11.50 ± 2.55	11.77 ± 1.21
K01 (min^{-1})	0.056 ± 0.027	0.040 ± 0.006
K10 (min^{-1})*	0.0076 ± 0.0014	0.0044 ± 0.0007
Tmax (min)	56.61 ± 14.05	71.45 ± 11.26
Cmax (ng/ml)	1759.17 ± 154.83	2013.40 ± 229.31
K01HL (min)	27.30 ± 13.25	19.87 ± 3.21
K10HL (min)	100.13 ± 17.74	267.9 ± 127.71
AUC[1] (ηg•min/ml•1000)	278.75 ± 40.60	349.67 ± 43.91
ONDANSETRON (20 mg/kg)		
PARAMETER	**PHASE 1**	**PHASE 2**
Volume (l/kg)	13.28 ± 2.30	12.38 ± 0.98
K01 (min^{-1})*	0.032 ± 0.005	0.059 ± 0.077
K10 (min^{-1})*	0.0093 ± 0.0007	0.0060 ± 0.0011
Tmax (min)	58.55 ± 6.00	47.54 ± 4.63
Cmax (ng/ml)*	964.22 ± 125.13	1284.85 ± 80.53
K01HL (min)*	26.40 ± 6.23	13.06 ± 1.67
K10HL (min)	76.62 ± 6.03	157.12 ± 42.97
AUC[1] (ηg•min/ml•1000)*	145.00 ± 18.00	207.13 ± 14.08
ONDANSETRON (10 mg/kg)		
PARAMETER	**PHASE 1**	**PHASE 2**
Volume (l/kg)	10.46 ± 1.51	10.83 ± 1.62
K01 (min^{-1})	0.035 ± 0.009	0.035 ± 0.008
K10 (min^{-1})	0.0113 ± 0.0017	0.0082 ± 0.0019
Tmax (min)	55.33 ± 5.55	66.07 ± 8.26
Cmax (ng/ml)	573.80 ± 67.86	631.64 ± 94.16
K01HL (min)	28.19 ± 5.61	30.49 ± 7.81
K10HL (min)	72.96 ± 11.86	120.22 ± 26.42
AUC[1] (ηg•min/ml•1000)	80.37 ± 11.77	101.75 ± 14.13

Phase 1 = parameter estimates of OND alone; Phase 2 = parameter estimates of OND in the presence of PYR. * Significant difference in parameter estimate between Phases ($p < 0.05$). Data are mean ± standard error of the mean.

(correlation coefficients = 0.9611, Phase 1; 0.9926, Phase 2) as a function of dose. The observations indicate that within the dosage range employed in

these studies, OND displayed linear pharmacokinetics in the absence (Phase 1) and presence (Phase 2) of PYR. For OND 10 mg/kg there were no significant differences in the mean parameter estimates between Phase 1 and Phase 2. The 20 mg/kg dose demonstrated several statistically significant differences between the two phases for the mean parameter estimates K_{01}, K_{10}, C_{max} $K_{01}HL$ and AUC ($p < 0.05$). The difference in estimates, relative to Phase 1 (Phase 2 - Phase 1/Phase 1), expressed as a percentage, was K_{01} 84%, K_{10} -35.5%, C_{max} 33%, $K_{01}HL$ 50.5% and AUC 43%. For 30 mg/kg the K_{10} was found to be significantly smaller in Phase 2 than Phase 1 ($p < 0.05$). The difference in K_{10} between Phase 1 and Phase 2 was calculated to be -42%. Model-predicted pharmacokinetic curves (Phase 1 compared to Phase 2) for the mean parameter estimates are presented in Figure 1 for doses 10 mg/kg (A), 20 mg/kg (B) and 30 mg/kg (C).

Results from these studies suggest that the presence of PYR produced changes in the pharmacokinetic parameter estimates of OND at the 20 and 30 mg/kg dose level. Therefore an attempt to eliminate or minimize this interaction was made by administering the compounds at different times. For this study, OND (20 mg/kg) was orally administered 150 minutes after pyridostigmine (Phase 3). A comparison of the pharmacokinetic parameters from Phase 1, 2 and 3 for OND 20 mg/kg are presented in Table 2.

Table 2: Comparison of the Mean Pharmacokinetic Parameters for Ondansetron 20 mg/kg; Phases 1, 2 and 3.

PARAMETER	PHASE 1	PHASE 3	PHASE 2
Volume (l/kg)	13.28 ± 2.30	12.49 ± 0.9	12.38 ± 0.98
K01 (min^{-1})	0.032 ± 0.005	0.046 ± 0.010	0.059 ± 0.007
K10 (min^{-1})	0.0093 ± 0.0007	0.0074 ± 0.0009	0.0060 ± 0.0011
Tmax (min)	58.55 ± 6.00	54.14 ± 4.7	47.54 ± 4.63
Cmax (ng/ml)*	964.22 ± 125.13	1095.34 ± 46.34	1284.85 ± 80.53
K01HL (min)	26.40 ± 6.23	18.56 ± 3.36	13.06 ± 1.67
K10HL (min)	76.62 ± 6.03	101.61 ± 11.08	157.12 ± 42.97
AUC[1] (ng•min/ml •1000)*	145.00 ± 18.00	169.28 ± 12.12	207.13 ± 14.08

Phase 1 = parameter estimates of OND alone; Phase 2 = parameter estimates of OND in the presence of PYR; Phase 3 = OND administered 150 minutes after PYR.
* Phase 1 = Phase 3; Phase 3 = Phase 2; Phase 1 = Phase 2.
All data are mean ± standard error of the mean.

Statistical analysis revealed that only the C_{max} and AUC demonstrated differences between the phases ($p < 0.05$); that is, Phase 1 = Phase 3, Phase 3 = Phase 2, Phase 1 = Phase 2 (see Table 3). For the rest of the parameters, no statistical differences could be distinguished. However, the data (Table 2) and the model-predicted curves (Figure 1D) demonstrated that the estimates, except volume, for Phase 3 lie between those for the Phase 1 and Phase 2 experiments. The trend in these data suggest that the change from Phase 1 to Phase 2 has been partially reversed by administering the drugs 150 min apart.

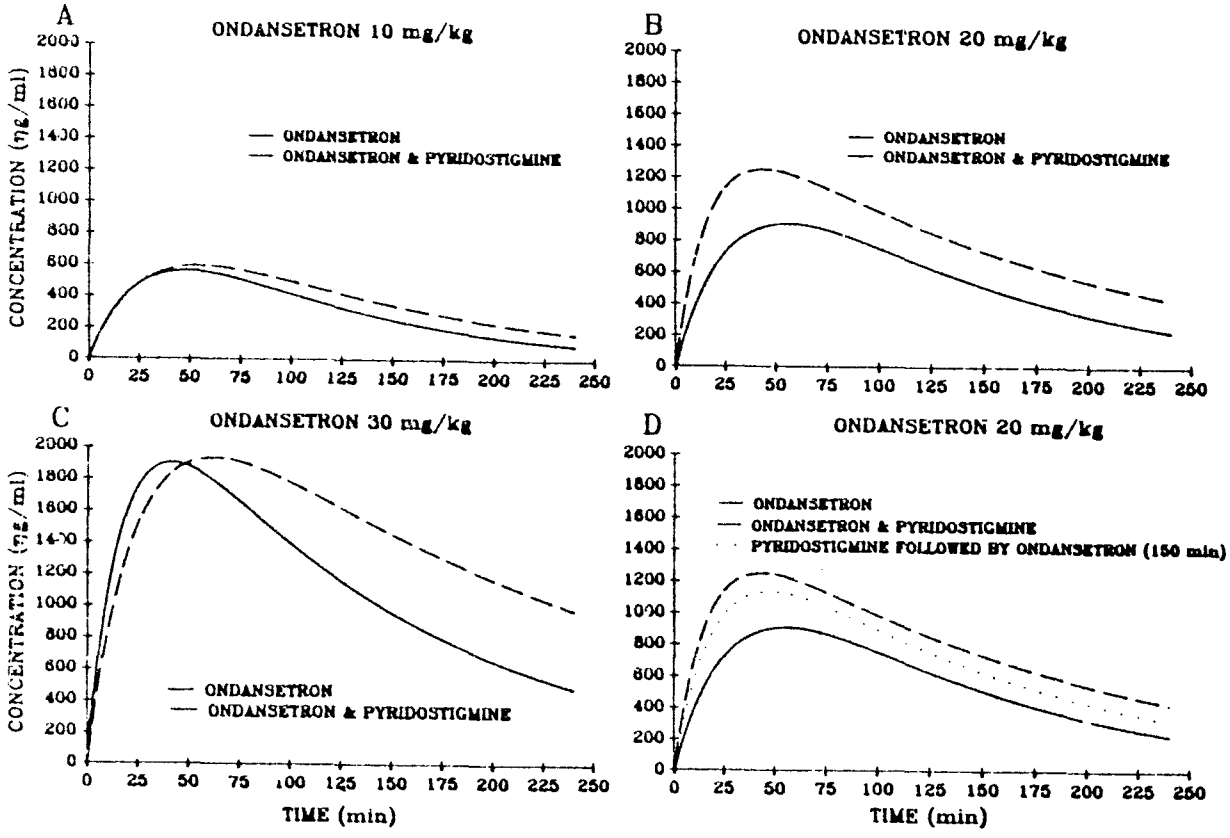

Figure 1. A,B,C - Ondansetron 10,20,30 mg/kg - Model-predicted pharmacokinetic curves in the absence (Phase 1) and presence (Phase 2) of PYR (0.94 mg/kg); mean of individual animal parameter estimates ($n \geq 6$). D - Ondansetron 20 mg/kg - Model-predicted pharmacokinetic curves; mean of individual animal parameter estimates; OND alone (Phase 1), with PYR (0.94 mg/kg) (Phase 2); and PYR (0.94 mg/kg) followed 150 min by OND (Phase 3)($n = 7$).

AChE Inhibition

AChE inhibition for the various treatment paradigms is presented in Figure 2 (A-D). Ondansetron (10 mg/kg) alone produced greater RBC AChE inhibition at 20, 30, and 240 min (12.6%, 12.0%, and 15.3%, respectively) when compared to water control at ($p < 0.05$) (Figures 1 and 3). The simultaneous administration of OND at 10 and 20 mg/kg with PYR produced significant additional AChE inhibition than that exhibited by either compound alone from 30 through 240 min. The additional inhibition ranged from 7.3%-17.2% for 10 mg/kg, and 14.2%-20.2% for the 20 mg/kg dose of OND plus PYR, with respect to PYR control (Figure 2, B and C). The additional effect on RBC AChE inhibition exhibited by the simultaneous administration of PYR and OND (10 and 20 mg/kg) was eliminated by separating the dosing times by 150 min (Figure 2D).

DISCUSSION

In this study the potential interactive effects of the two drugs PYR and OND (after concurrent oral administration) have been assessed in guinea pigs. The oral pharmacokinetics of OND in the absence and presence of PYR were demonstrated to be linear in the dose range examined. We have observed that the apparent volume of distribution for OND in these experiments (approximately 12-13 L/kg) is relatively large. The magnitude of the apparent volume is the result of small plasma concentrations relative to the dose administered. This suggests that the drug is being removed from the plasma to other tissue compartments (12), thus, effectively reducing the plasma concentrations in this species. Saynor and Dixon (13) have noted that the bioavailability of OND after oral administration in animals is < 10%, due to extensive first-pass hepatic metabolism. Therefore, it is possible that this metabolic effect is, in part, responsible for the large apparent volume of distribution. Absorption of OND from the gastrointestinal tract of rats and dogs has been shown to be rapid (i.e., T_{max} approximately 40 min) (13). This is in the same range as our experiments where the drug demonstrated a T_{max} of 55-58 minutes. In contrast to the findings of Saynor and Dixon (13) who reported relatively short half-lives in dogs and rats (10 and 30 min respectively) we have noted a longer half-life of elimination ($K_{10}HL$ = 72 to 100 min). This discrepancy may be due to a number of differences including species and/or dose.

Examination of the pharmacokinetic data for OND in the presence of PYR demonstrated that the PYR had some effects on OND parameter estimates (Table 1). The addition of PYR consistently decreased K_{10} and increased C_{max}, AUC and $K_{10}HL$ at all three doses (see Table 1). These data suggest that the decreases in the plasma elimination rate of OND produced increases the C_{max} and AUC. Although we have noted changes in the pharmacokinetic parameters of OND in the presence of PYR, the significance of the findings with respect to OND safety and efficacy in any species is uncertain.

The primary route for OND metabolism in rat and man has been reported to be hydroxylation by microsomal cytochrome P-450 metabolizing enzymes followed by glucuronide or sulphate conjugation (13). Other routes involving N-demethylation followed by hydroxylation have also been reported to be major in the dog and minor in the rat and man by the same authors. The metabolic disposition of PYR has been the subject of much controversy. It is generally agreed that the primary metabolite of PYR, due to hydrolysis, is 3-hydroxy-N-methyl pyridinium (3-HMP) (14-17). Although the presence of other metabolites has been reported, the identity of these compounds has been somewhat unclear (13-17). Kornfeld *et al.* (14) have proposed a number of metabolites of PYR resulting from conjugation (glucuronides) and demethylation reactions (demethylated glucuronides). Somani *et al.* (15) have indicated the existence

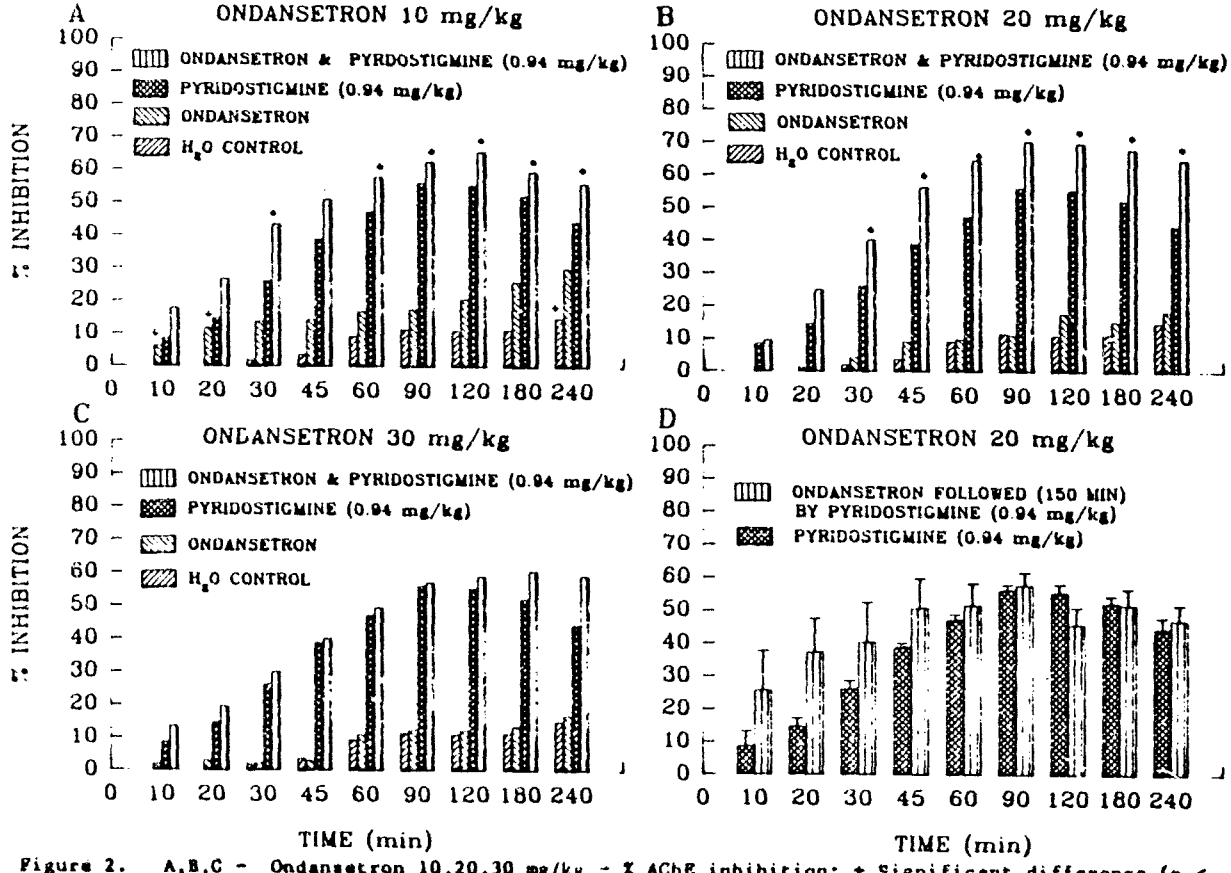

Figure 2. A,B,C - Ondansetron 10,20,30 mg/kg - % AChE inhibition; + Significant difference ($p < 0.05$) OND vs H_2O control ($n = 6$); * Significant difference ($p < 0.05$) OND plus PYR vs PYR control. D - % AChE inhibition; no significant differences between PYR control and OND followed by PYR (150 min) ($n = 6$).

of a dihydroxy-N-methyl pyridinium metabolite resulting from hydroxylation of 3-HMP. In addition, Birtley et al. (13) have suggested demethylation as a possible minor route for PYR disposition. Moreover, the related quaternary AChE inhibitor neostigmine has been shown to undergo conjugation reactions (18) and the monoglucuronide conjugate has been isolated. In soluble liver fractions in vitro, PYR is hydrolyzed to 3-HMP (17,19). The liver has been suggested to be the main site of PYR metabolism in rats (14). Barber et al. (20) have shown that PYR is subject to metabolism during the first passage through the liver. Also, the initial product resulting from carbamylation-decarbamylation of cholinesterase is 3-HMP. Thus, there exist at least two potential mechanisms for PYR hydrolysis. In addition, however, there is evidence to suggest that other metabolizing enzyme systems may also be involved in the biotransformations of PYR. As far as the interaction observed between PYR and OND, the available literature indicates that PYR or possibly its metabolite(s) have effects on the biotransformation of OND in the liver. It is possible that this occurs at a point where the two compounds undergo a common metabolic conversion (i.e., hydroxylation, conjugation or demethylation). Finally, it should be mentioned that the physiologic significance of these findings is unclear.

Ondansetron alone did not significantly modify AChE inhibition except for 10 mg/kg (20, 30, and 240 min). We believe, however, that this is an artifact, due to the small group variance relative to the other treatments in this phase, and may not be due to a direct inhibition of cholinesterase by OND. The concurrent administration of OND with PYR produced an increase in AChE inhibition by 10-20% for OND 10 and 20 mg/kg beyond the 30-minute time point relative to PYR control (Figure 2). This additional effect may be due to the changes in mouth to cecum transit time (21) or enhanced gastro-intestinal motility, reportedly induced by 5-HT_3 inhibitors (6,22) to alter PYR absorption.

For the Phase 3 experiments, the pharmacokinetic estimates followed a consistent trend. That is, the Phase 3 estimates, except for volume, were between those for the Phase 1 and Phase 2 (Table 2). This can be further seen by inspection of the model predicted curves (Figure 1D). The difference in administration times between PYR and OND (150 min) in our experiments represents approximately six half-lives for PYR elimination in rats (23). It appears that this time differential is sufficient to allow most of the administered PYR to be cleared from the plasma and offset the interaction resulting from simultaneous administration of the two drugs. Similarly, the increased inhibitory effect on PYR-induced RBC AChE activity was eliminated by separating the administration times of the drugs by 150 minutes (approximately two half-lives for OND elimination).

In summary, the simultaneous oral administration of PYR and OND resulted in changes in the pharmacokinetic parameters of OND and an additional increase of PYR-induced AChE inhibition. These two effects could be reversed by administering the compounds 150 min apart.

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